



## Full Length Article

# Use of Fourier transform infrared spectroscopy (FTIR) spectroscopy for rapid and accurate identification of Yeasts isolated from human and animals

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## KEYWORDS

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**Abstract** Rapid and accurate identification of yeast is increasingly important to stipulate the appropriate therapy thus reducing morbidity and mortality related to yeast infections. Vibrational spectroscopic techniques (infrared (IR) and Raman) could provide potential alternatives to conventional typing methods, because they constitute a rapid, inexpensive and highly specific spectroscopic fingerprint through which microorganism can be identified. The present study evaluate (FTIR) spectroscopy as a sensitive and effective assay for the identification of the most frequent yeast species isolated from human and animals. One hundred and twenty-eight yeasts isolated from infected human mouths/vaginas, chronic diseased cows, crop mycosis in chicken and soil contaminated with pigeon droppings were phenotypically identified. Using universal primers, ITS1/ITS4, we have amplified ITS1-5.8S-ITS2 rDNA regions for 39 yeast isolates as representative samples. The PCR products were digested with restriction enzyme *MspI* and examined by PCR-RFLP, which was an efficient technique for identification of *Candida* spp., *Cryptococcus neoformans* and *Trichosporon asahii*. Further, identification of the same 39 isolates were done by FTIR spectroscopy and considered as reference for other strains by comparison of their FTIR spectra. The current study has sharply demonstrated the significant spectral differences between the various examined species of *Candida*, *Cryptococcus*, *Trichosporon*, *Rhodotorula* and *Geotrichum* isolated from different sources. Decisively, our research has confirmed that FTIR spectroscopy is a promising diagnostic tool, because of its sensitivity, rapidity, high differentiation capacity and simplicity compared to conventional/molecular techniques.

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## 1. Introduction

Classical identification of yeasts can be achieved by applying physiological and morphological tests to determine enzyme production profiles and growth characteristics [2]. Despite the fact that conventional/classical tests are largely applied, yet, these tests are faced with several constraints. Time consuming and low discriminative power between closely related species are the major constraints [16].

Currently, genotypic methods are practically preferred than phenotypic methods. The following molecular assays are the most current molecular techniques used for identifying yeast strain identity: randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism analysis (RFLP), multilocus enzyme electrophoresis (MEE), DNA sequencing, and pulsed-field gel electrophoresis [13]. However, their application in the routine analysis is limited by their protocol complexities, reagent costs, choice of specific primers for each species, sensitivity to mutations and the requirement for highly skilled personnel [4,17].

Most recently, an approach that is based on optical spectroscopic techniques as FTIR spectroscopy has been proposed for identification of microorganisms. FTIR spectra provide highly specific spectroscopic fingerprints of microorganisms allowing an accurate identification at both species and sub-species levels [8,11]. This approach represents an analytical, nondestructive, and dynamic method to investigate a cell population with little biomass [11].

FTIR spectroscopy is based on the vibrational excitation of molecular bonds by absorption of infrared light energy (only the middle infrared section). The sum of vibrational spectra for a cell macromolecule (nucleic acids, proteins, lipids, polysaccharides, etc.) can produce an infrared absorption spectrum that looks like a molecular “fingerprint” for such biological material. This spectrum can be used in typing or identification applications through clustering [5]. Fortunately, a reference spectrum library is assembled based on well-characterized strains and species. The FTIR spectrum of any unidentified isolate can be measured under the same conditions as those used for the reference spectra and then compared to different spectra from the reference spectrum library. If the library contains an identical or a very similar spectrum, identification is possible. The success of the method is, therefore, directly dependent on the complexity of the reference spectrum library [7].

In the present study, the potential of FTIR spectroscopy for typing yeast strains isolated from human and animals was tested. Furthermore, the typing potential of FTIR spectroscopy was compared to that of the well established molecular tool, RFLP.

## 2. Materials and methods

### 2.1. Isolation and identification of yeasts from human and animals

One hundred and twenty-eight yeasts were isolated from a total of 211 samples collected from human oral/vaginal candidosis (20 swabs/each), chronic diseased cows (126 samples including 21 rectal, 20 vaginal, 26 oral, 19 nasal, 20 ear swabs and 20 mastitic milk sample), crop mycosis in chicken (20 swabs) and soil contaminated with pigeon droppings (25 sam-

ples). All yeast isolates were fully identified according to macro-morphology on Sabouraud Dextrose Agar (SDA, Difco Lab., USA) with chloramphenicol, micro-morphology (hyphae, pseudohyphae, chlamydospores, arthrospores) on rice agar media and chromogenic candida agar (CCA, Difco Lab., USA) for *Candida* spp. [15].

### 2.2. Identification of yeast species based on RFLP of the 5.8S-ITS region

#### 2.2.1. DNA extraction

Genomic DNA of a total of 39 yeast representative isolates was extracted using the Gene Jet Genomic DNA purification kit K0721, K0722 (Fermentas, Germany).

#### 2.2.2. PCR

PCR amplification of ITS1-5.8SITS2 rDNA regions was achieved using region specific primers: ITS1 (forward, 5'-TCC GTA GGT GAA CCT GCG G-3' and ITS4 (reverse, 5'-TCC TCC GCT TAT TGA TAT GC-3') primer pairs (Fermentas, Germany) after [10,14]. The amplification steps consisted of 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 56 °C for 1 min., extension at 72 °C for 1 min. In the first cycle, the denaturation step was 94 °C for 5 min. and in the final cycle the final extension step was 72 °C for 7 min.

#### 2.2.3. Restriction enzyme analysis

For restriction digestion, 21.5 µl of PCR products were digested directly and individually by 1 µl of the restriction enzyme *MspI* and 2.5 µl related buffer (total reaction volume of 25 µl) after 3 h incubation at 37 °C. The PCR products and their restriction fragments were separated on 1.5% agarose gels containing 0.5% ethidium bromide in 1×-Tris Acetate EDTA (TAE) buffer at 100v and their sizes estimated by comparison against a 100 bp DNA ladder. DNA was visualized under UV trans-illuminator and gel image analysis was done by gel documentation system.

### 2.3. Identification of yeasts by FT-IR spectroscopy

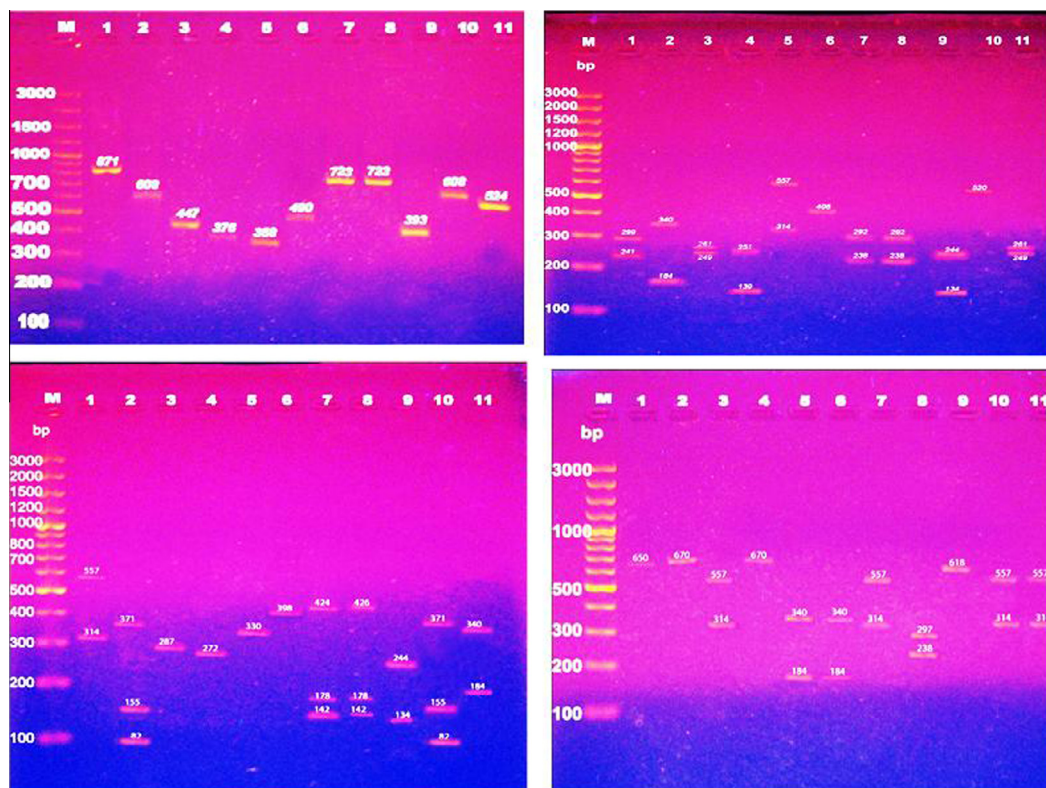
A total of 39 *Candida* isolates formerly identified by phenotypic and RFLP methods, were analyzed using FTIR spectroscopy.

#### 2.3.1. Sample preparation for FT-IR spectroscopy

Potassium bromide (KBr) based pellets were prepared by establishing pressure of 10 kg/cm<sup>2</sup> for about 30 s. A pure KBr tablet was used as a blank for background subtraction. Loopfuls from yeast isolates cultured on SDA supplemented with chloramphenicol were taken and placed on KBr discs.

#### 2.3.2. FT-IR spectral measurement

All spectra (61 spectra) ranging between wave numbers 4000 and 400 cm<sup>-1</sup> were recorded with Fourier transform infrared spectroscopy (Jasco FTIR 6100 Japan). For data processing, the software OPUS (version 2.2) was used for microbiological identification (Bruker). All spectra were base line corrected and vector normalized. Spectral resolution was set at 4 cm<sup>-1</sup> [12].



**Plate 1** Agarose gel electrophoresis showing size of the PCR-amplified rDNA region of *Candida* isolates (A) and restriction analysis with *MspI* (B–D). Plate 1 (A) Lane M, molecular size marker; Lane 1, *C. glabrata*; Lanes 2 and 10, *C. guilliermondii*; Lane 3, *C. inconspicua*; Lane 4, *C. lusitaniae*; Lane 5, *C. lipolytica*; Lane 6, *C. vini*; Lanes 7 and 8, *C. kefyr*; Lane 9, *C. catenulata* and Lane 11, *C. tropicalis*. (B) Lane M, molecular size marker. Lane 1, *C. utilis*; Lane 2, *C. tropicalis*; Lanes 3 and 11, *C. krusei*; Lane 4, *C. norvegensis*; Lane 5, *C. glabrata*; Lane 6, *C. lambica*; Lanes 7 and 8, *C. albicans var stellatoidea*; Lane 9, *C. catenulata* and Lane 10, *C. parapsilosis*. (C) Lane M, molecular size marker, Lanes 1–11, *C. glabrata*, *C. guilliermondii*, *C. inconspicua*, *C. lusitaniae*, *C. lipolytica*, *C. vini*, *C. kefyr*, *C. catenulata*, *C. guilliermondii* and *C. tropicalis*. (D) Lane M, molecular size marker; Lane 1, *C. humilis*; Lanes 2 and 4, *C. peltata*; Lanes 3, 7, 10 and 11, *C. glabrata*; Lane 5 and 6, *C. tropicalis*; Lane 8, *C. albicans* and Lane 9, *C. saitoana*.

Different spectra from the same isolates were compared together. When they showed identical curves, considered as reference curve. The same *Candida* species according to phenotypic/PCR results were compared with that reference curve. Finally, reference curves of all isolated species in this study were compared together and spectral data library were established. Measurement and comparison of the spectrum with the reference spectrum library containing spectra of defined strains could take less than 2 min.

### 2.3.3. Cluster analysis

Hierarchical cluster analysis (HCA) was applied to compare samples and grouping the spectra with the same degree of similarity. The method calculates the Euclidean distance between all the data set by using the Ward's algorithm method. The merging process can be visualized in a tree-like diagram, called a dendrogram, presenting the regrouping of the spectra in clusters according to a heterogeneity scale [7,17].

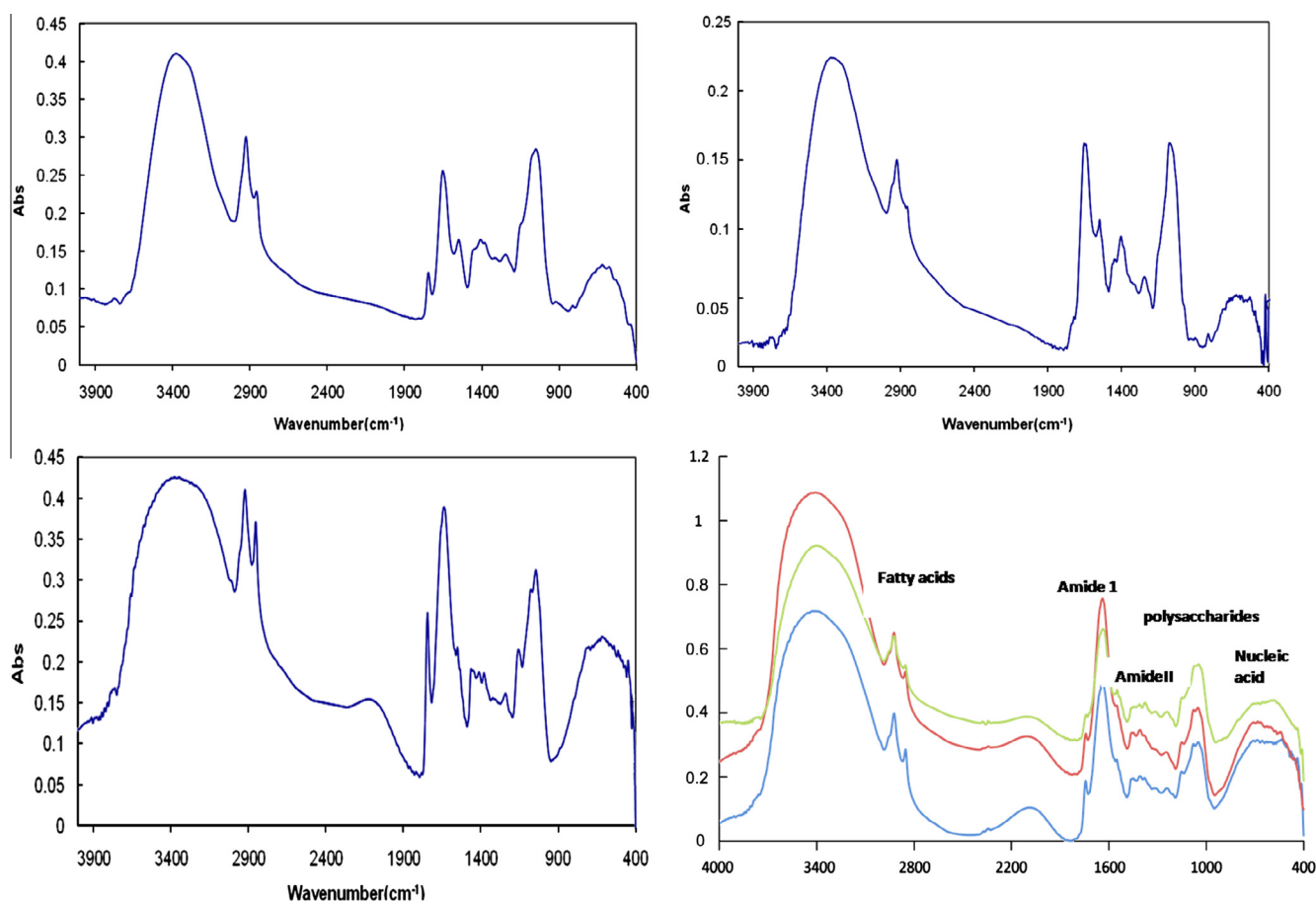
## 3. Results

The highest percentage of *Candida* spp. isolated from human oral and vaginal candidosis was reported for *Candida albicans*

(30%) followed by *Candida tropicalis* (5%) then *Candida parapsilosis*, *Candida glabrata* (2.5%) for each three *Candida* spp., that were further identified by PCR-RFLP into *Candida lambica*, *Candida catenulata* and *Candida utilis*. In respect to cow samples, a total of 53 *Candida* isolates were retrieved. The most common *Candida* spp. were *Candida krusei* (35.8%) followed by *C. albicans* (28.3%) then *C. parapsilosis* (11.3%), *C. glabrata* (7.5%), *C. tropicalis* (5.6%) and five *Candida* spp., were further identified by RFLP into *Candida guilliermondii* (3.7%), *Candida norvegensis*, *Candida humilis*, *Candida peltata* and *C. catenulata* (1.8%) for each. From mastitic milk samples, *Candida lipolytica*, *Candida vini*, *Candida inconspicua*, *C. peltata*, *C. albicans* and *Rallina rubra* were isolated. *C. albicans* (60%) was the most common *Candida* spp. isolated from crop mycosis followed by *C. glabrata* (20%) then *C. krusei* (10%) and *Candida lusitaniae* (10%). A total of five isolates (20%) of *Cryptococcus neoformans*, four *Trichosporon* spp. (16%), nine *Geotrichum candidum*, four *R. rubra*, two *Candida kefyr* and one isolate *Candida saitoana* were retrieved from soil contaminated with pigeon droppings.

In this study, RFLP was run for the sake of identification of *Candida* spp., *Cryptococcus neoformans* and *Trichosporon* spp., using universal primers ITS1 and ITS4 amplified ITS region. The reaction has yielded a unique PCR product size of





**Plate 2** (A) FT-IR spectrum of *C. albicans*. (B) FT-IR spectrum of *C. neoformans*. (C) FT-IR spectrum of *T. asahii*. (D). Identical absorption spectra of three *C. tropicalis* strains with some characteristic spectral ranges that are dominated by certain chemical structures: fatty acids ( $3050\text{--}2800\text{ cm}^{-1}$ ); amide I ( $1600\text{--}1700\text{ cm}^{-1}$ ), amide II ( $1500\text{--}1600\text{ cm}^{-1}$ ), polysaccharides ( $1200\text{--}900\text{ cm}^{-1}$ ) and nucleic acid ( $700\text{--}900\text{ cm}^{-1}$ ).

approximately 358–871 bp. The approximate length of the amplified products was enough to identify the species *C. glabrata* (871 bp), *C. kefir* (723 bp), *C. pelata* (680 bp), *C. humilis* (670 bp), *C. saitoana* 614 bp, *C. guilliermondii* (608 bp), *Candida stellatoidea* (560 bp), *C. utilis* (555 bp), *C. albicans* (535 bp), *C. tropicalis* (524 bp), *C. parapsilosis* (520 bp), *C. krusei* (510 bp), *C. vini* (490 bp), *C. norvegensis* (488 bp), *C. inconspicua* (447 bp), *C. lambica* (432 bp), *C. catenulata* (393 bp), *C. lusitaniae* (376 bp), *C. lipolytica* (358 bp), *C. neoformans var neoformans* (556 bp), *C. neoformans var grubi* (550 bp) and *Trichosporon asahii* (515 bp) (Plate 1A).

PCR amplicons were digested with enzyme *MspI* and the generated bands corresponded to the predicted sizes. *MspI* resulted in DNA cleave, where there is a CCGG sequence. (Plate 1B–D).

Spectral variations were observed within different yeasts that were previously identified by phenotypic/PCR methods as in Plate 2A–C. The resultant spectra for these yeasts were used as private library data base (reference for different species). Spectra of different samples from the same species showed great similarity and matching (100%) with the original library data base as in Plate 2D.

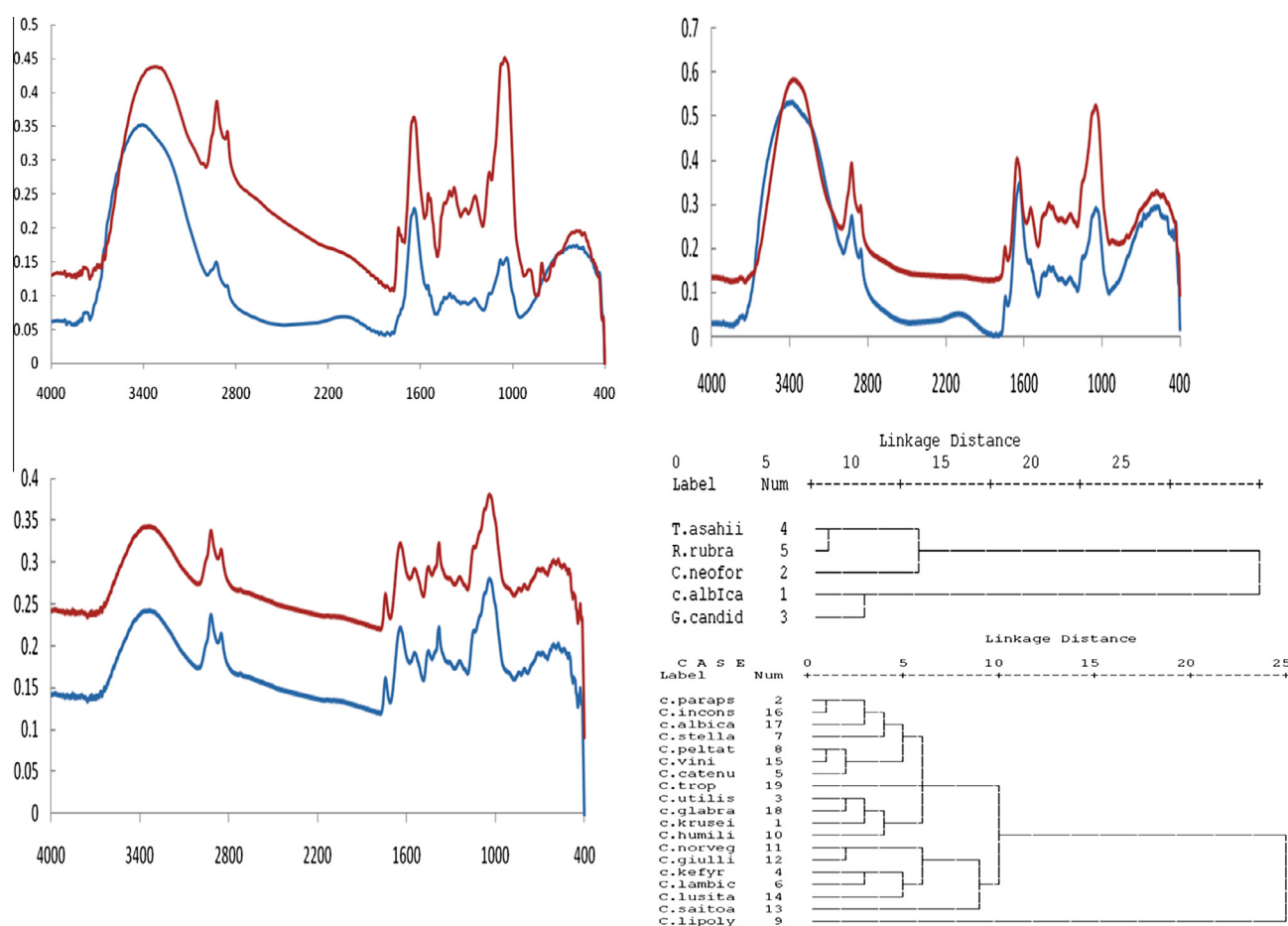
There are clear spectral differences between reference curves of different species (Plate 3A and B). When six un-

known tested samples were identified by FTIR spectroscopy and their spectra were compared with reference curves, they looked identical as shown in Plate 3C.

Cluster analysis with Ward's algorithm using three spectral windows ( $3030\text{--}2830$ ,  $1350\text{--}1200$ , and  $900\text{--}700\text{ cm}^{-1}$ ) allowed differentiating yeast genera into two clusters (Plate 3D1) which corresponded to the basidiomycetous and ascomycetous yeasts, respectively. Further, *Candida* spp., could be clearly differentiated and infrared spectra of closely related species can be pooled together showing a complete concordance (e.g. *C. albicans* and *C. stellatoidea*) (Plate 3D2).

#### 4. Discussion

Phenotypic examination and PCR were the most acceptable methods for identification of yeasts. However, phenotypic methods are not fast and reliable enough, and PCR is expensive. Recently, use of FTIR spectroscopy for identification of yeasts has significantly increased [5,13]. In present study, our objective was to investigate the capability of FTIR spectroscopy technique to identify and discriminate between yeasts isolated from human and animals. For this purpose, 120 yeast isolates were identified by phenotypic method, RFLP and FTIR spectroscopy analyses.



**Plate 3** (A) Absorption spectra of *R. rubra* and *R. glutinis*. Difference in nucleic acid, polysaccharides and carbonyl region of lipids. (B) Absorption spectra of *C. albicans* var *stellatoidea* and *C. albicans*. Difference in nucleic acid region. (C) FTIR spectra of unknown sample seem looked identical to *C. parapsilosis*. (D1) Dendrogram of examined yeast genera, (D2) Dendrogram showing the hierarchical clustering of 19 *Candida* species considering the spectral ranges from 3030 to 2830, 1350 to 1200, and 900 to 700  $\text{cm}^{-1}$ .

RFLP system was developed for the identification of clinically relevant yeast species. By RFLP, used primers could amplify DNA from all 39 tested yeasts including *Candida*, *Cryptococcus* and *Trichosporon*, which represent a broad range of clinically relevant yeasts. The primer provided an amplicon (358–871 bp) from all tested isolates. This result was in agreement with previously published reports [1,11,16]. Authors have successfully identified only six *Candida* spp. using RFLP and fungus-specific universal primers (ITS1 and ITS4) and then PCR amplicons were digested with *MspI*, while Shokohi et al. [14] digested the PCR products with two restriction enzymes *MspI* and *BlnI* separately. In the present study, 19 *Candida* species, two *C. neoformans* and one *Trichosporon* spp. could be identified using ITS1 and ITS4 primers with restriction enzymes *MspI*. Fujita et al. [6] identified *Candida* spp. by multiplex PCR using internal transcribed spacer 1 and 2 regions.

*MspI* could potentially distinguish 22 yeast species. Nine *Candida* species with a single restriction digest were obtained. While Mirhendi et al. [9,10] obtained the same result for identification of *Candida* spp. by chromogenic candida agar and RFLP methods. In the present study, chromogenic candida agar could not identify 12 isolates of *Candida* spp. Complete

identification of these isolates were obtained by RFLP. This result suggests that the selection of chromogenic candida agar media is controversy. This in agreement with [6,14]. Analysis of RFLP derived from the DNA of *Candida* spp. has the advantage of being reliable in comparison with the phenotypic method, which is insensitive and have limited availability [3].

The FTIR spectra exhibited well defined spectral regions that correspond to the vibration of a given chemical entity and hence to a constituent of the yeast cell. The main observed features were characteristic vibrations, which occurred due to the peptide bond of proteins (Amide I in the 1600–1700  $\text{cm}^{-1}$  and Amide II in the 1480–1600  $\text{cm}^{-1}$ ), nucleic acids PO2 (1180–1300  $\text{cm}^{-1}$ ), carbohydrates (900–1200  $\text{cm}^{-1}$ ), and (900 to 700  $\text{cm}^{-1}$ ) contains bands which are most characteristic at the species level [11].

Based on this result, we recommend FTIR spectroscopy as a candidate to identify *Candida*, *C. neoformans*, *Trichosporon*, *Rhodotorula* and *Geotrichum* spp. most frequently encountered in human and animal infections. To that end 39 yeast isolates were investigated by phenotypic, RFLP and FTIR spectroscopy method and their spectra considered as reference to which other species were compared with its corresponding in our library. All isolates have showed great identity and

matching with their reference curves. This rate of matching between the obtained curves and the reference curves of the same species in this study library and lack of similarity between different isolated species reinforced the fact that FT-IR spectroscopy technique provides spectral fingerprints for each fungal species. Similarly, Sandt et al. [13] demonstrated that FT-IR spectroscopy is potent enough to identify *C. albicans* with a high sensitivity. FT-IR microspectroscopy is able to identify microcolonies of *Candida* species [4]. Moreover, Essendoubi et al. [5] typed *C. glabrata* clinical isolates by FTIR spectroscopy. Also Fujita et al. [6] demonstrated the feasibility of the FT-IR technique for intraspecies comparison of three *Candida* species (*C. albicans*, *C. glabrata* and *C. parapsilosis*). Compared to other reported data, the present study has strong points in the sense that a more important number of yeast species were analyzed using FT-IR spectroscopy. The results showed that the spectral windows with the best discriminating features were localized in the region between 3030–2830, 1350–1200, and 900–700  $\text{cm}^{-1}$ , which coincides with many previous literatures [7,17].

FT-IR spectra can reflect small variations due to culture parameters (e.g., culture age, medium composition, medium pH, temperature, humidity, and storage mode). As a result, only spectra recorded for the same batch of culture medium, at the same temperature, and during the same precise culture time were compared. Thus, each model has to be standardized and fixed in regards to species, culture and sample preparation conditions for a better spectral reproducibility [13,4].

## 5. Conclusion

FTIR spectroscopy is a simple, rapid, less expensive and highly sensitive tool for identification as well as differentiation between different genera and species of yeasts.

## References

- [1] Allam AA, Salem IM. Evaluation of rapid molecular identification of clinically important *Candida* spp. isolated from immuno-compromised patients using RF-PCR. *J Am Sci* 2012;8:463–8.
- [2] Barnett JA, Payne RW, Yarrow D. Yeasts: characteristics and identification. Cambridge, United Kingdom: Cambridge University Press; 2000.
- [3] Cirak YM, Kalkanci A, Kustimur S. Use of molecular methods in identification of *Candida* species and evaluation of fluconazole resistance. *Mem Inst Oswaldo Cruz, Rio de Janeiro* 2003;98:1027–32.
- [4] Essendoubi M, Toubas D, Bouzaggou M, Pinon JM, Manfait M, Sockalingum GD. Rapid identification of *Candida* species by FT-IR microspectroscopy. *Biochim Biophys Acta* 2005;1724:239–47.
- [5] Essendoubi M, Toubas D, Lepouse C, Leon A, Bourgeade F, Pinon JM, et al. Epidemiological investigation and typing of *Candida glabrata* clinical isolates by FTIR spectroscopy. *J Microbiol Methods* 2007;71:325–31.
- [6] Fujita S, Senda Y, Nakaguchi S, Hashimoto T. Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. *J Clin Microbiol* 2001;39:3617–22.
- [7] Kümmerle M, Scherer S, Seiler H. Rapid and reliable identification of food-borne yeasts by Fourier-transform infrared spectroscopy. *Appl Environ Microbiol* 1998;64:2207–14.
- [8] Maquelin K, Kirschner C, Choo-Smith LP, Ngo-Thi NA, Van Vreeswijk T, Stammler M, et al. Prospective study of the performance of vibrational spectroscopies for rapid identification of bacterial and fungal pathogens recovered from blood cultures. *J Clin Microbiol* 2003;41:324–9.
- [9] Mirhendi H, Makimura K, Zomorodian K, Maeda N, Ohshima T, Yamaguchi H. Differentiation of *C. albicans* and *C. dubliniensis* using a single-enzyme PCR-RFLP method. *Jpn J Infect Dis* 2005;58:235–42.
- [10] Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. A One-enzyme PCR-RFLP assay for identification of six medically important *Candida* species. *Jpn J Med Mycol* 2006;47:225–9.
- [11] Naumann D, Helm D, Labischinski H. Microbiological characterizations by FT-IR spectroscopy. *Nature* 1991;351:81–2.
- [12] Salem EZ, Shahin IM, Yaser FM, Hamed MA, Abdel Hamid MF, Emam H, et al. Applicability of Fourier transform infrared (FT-IR) spectroscopy for rapid identification of some yeasts and dermatophytes isolated from superficial fungal infections. *J Egypt Women Dermatol Soc* 2010;7:105–10.
- [13] Sandt C, Sockalingum GD, Aubert D, Lèpau H, Lepouse C, Jaussaud M, et al. Use of Fourier-transform infrared spectroscopy for typing of *Candida albicans* strains isolated in intensive care unit. *J Clin Microbiol* 2003;41:954–9.
- [14] Shokohi T, Sotekh HM, Ashemi, Pouri ZS, Hedayati MT, Mayahi S. Identification of *Candida* species using PCR-RFLP in cancer patients in Iran. *Indian J Med Microbiol* 2010;28:147–51.
- [15] Taha M. Medical mycology: atlas of important fungi and dermatomycosis. Cairo: Mecca Printing House; 2011.
- [16] Toubas D, Essendoubi M, Adt I, Pinon JM, Manfait IM, Sockalingum GD. FTIR spectroscopy in medical mycology: applications to the differentiation and typing of *Candida*. *Anal Bioanal Chem* 2007;387:1729–37.
- [17] Wenning M, Seiler H, Scherer S. Fourier-transform infrared microspectroscopy, a novel and rapid tool for identification of yeasts. *Appl Environ Microbiol* 2002;68:4717–21.